Pestic. Sci. 1998, **52**, 91–96

Consumption of Fipronil-Treated Rice Seed Does Not Affect Captive Blackbirds

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(Received 24 February 1997; revised version received 3 July 1997; accepted 27 August 1997)

Abstract: Fipronil is an insecticide developed for use on rice seed and other crops. In a series of cage and pen trials, we evaluated the responses to dyed, fipronil-treated rice seed of three bird species likely to encounter it in the field. Individually caged red-winged blackbirds (*Agelaius phoeniceus*), brown-headed cowbirds (*Molothrus ater*), and boat-tailed grackles (*Quiscalus major*) displayed no evidence of adverse reaction to treated seed. Chemical analyses of hulls from treated seeds eaten by captive birds revealed that 10–20% of the fipronil originally present was removed during feeding. In group enclosures, male red-winged blackbirds ate as much fipronil-treated rice as they did dyed, untreated seed. In four-day tests within a 0·2-ha flight pen, 10-bird blackbird flocks removed 11·4% of fipronil-treated seed from a test plot compared to 12·5% of dyed, untreated seed removed from the alternate plot. When the alternate plot contained undyed rice, however, seed removal from the treated plot averaged 2·4% compared to 28·9% from the alternative plot, suggesting that the groups of test birds avoided treated seed based on its appearance. We conclude that 325 and 500 mg kg^{-1} fipronil applications alone do not affect avian feeding activity. © 1998 SCI.

Pestic. Sci., **52**, 91–96, 1998

Key words: fipronil; seed treatment; rice; red-winged blackbird; color avoidance

1 INTRODUCTION

Fipronil (($\pm$)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile; Rhône-Poulenc) is an insecticide for seed, soil, and foliar applications. Seed treatment of rice with fipronil has produced good control of rice water weevil (*Lissorhoptrus oryzophilus* Kuschel), a major pest to rice.[1] This is particularly important given that carbofuran, the pesticide currently used to control rice water weevil, will not be available after 1997.[2] Thus, birds frequenting newly seeded rice fields are likely to be exposed to fipronil as use of fipronil-treated rice seed increases in the near future.

Fipronil belongs to the phenylpyrazole class of compounds which act on the nervous system of insects by blocking the receptor regulated by the neurotransmitter

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Contract grant sponsor: Rhône-Poulenc Ag Company
Contract grant sponsor: US National Wildlife Research Center

4-aminobutyric acid.[3] Available information (Rhône-Poulenc Ag Company, unpubl. rep.) suggests that fipronil has relatively low acute mammalian toxicity (rat oral $LD_{50} = 97$ mg kg^{-1}), and low acute avian toxicity to mallards, *Anas platyrhynchos* L. ($LD_{50} > 2150$ mg kg^{-1}), pigeons, *Columba livia* Gmelin (>2000 mg kg^{-1}) and field sparrows, *Spizella pusilla* Wilson (1120 mg kg^{-1}). Northern bobwhite, *Colinus virginianus* L. ($LD_{50} = 11{\cdot}3$ mg kg^{-1}), ring-necked pheasant, *Phasianus colchicus* L. (31 mg kg^{-1}) and red-legged partridge, *Alectoris rufa* L. (34 mg kg^{-1}) appear to be more sensitive, however.

During field trials, there were anecdotal reports of bird avoidance of rice seed treated with fipronil (M. O. Way, Texas A&M University, Beaumont, TX, pers. comm.). If verified, such bird deterrence would benefit growers in the south-eastern United States, where bird damage to newly seeded rice can cause major economic losses.[4,5] Other agricultural chemical seed treatments can reduce blackbird consumption of rice,[6,7] and in this study we evaluated, under a range of test conditions, the repellency of fipronil-treated rice seed to three species of birds likely to be exposed to the treatment in the field. Additionally, we assessed the potential for adverse impacts to granivorous birds by determining the amount of fipronil removed by birds during feeding, and we assessed the effect of a blue dye on seed acceptance.

2 METHODS

2.1 Experimental subjects

Male red-winged blackbirds (*Agelaius phoenicus* L), male and female brown-headed cowbirds (*Molothrus ater* Boddaert) and female boat-tailed grackles (*Quiscalus major* Vieillot) were trapped near Gainesville, Florida and held in captivity one to three months prior to testing. Female redwings and male grackles were not available in sufficient numbers to be tested. Birds were housed in communal cages (1·8 × 1·3 × 1·3 m) by species in a roofed outdoor aviary with free access to commercial game bird food (Hillandale Farms, Lake Butler, FL) and water.

2.2 One-cup feeding trials

The one-cup feeding trials were conducted with all three test species in a roofed outdoor aviary, where test cages (45 × 45 × 45 cm) were visually isolated and equipped with waterers. Food was presented in clear plastic feed cups (8·2 cm diameter, 3·8 cm high) with a circular opening (3·1 cm diameter) in the top.

Four days before the start of a trial, birds were taken from their holding cages, weighed, and randomly assigned to individual test cages to form three treatment groups of six birds each. During the four-day acclimation period, birds were given a mixture of untreated rice seed and commercial game bird food.

Following acclimation, there was a four-day pre-treatment period and a four-day treatment period. During pre-treatment, each cup contained 30 g of untreated, undyed rice seed. In the treatment phase, birds received dyed rice at one of three fipronil levels: 0, 325, or 500 mg kg^{-1}. To be used in the field, all pesticide-treated seed must include a dye as part of the formulation. Dyed rice looked bluish-green, and on a standard color chart most closely matched color 164, 'cyan', specified in Munsell notation as 5.3 BG 5.7/10.4.[8]

On test days, maintenance food was removed at 0800, and 1 h later the test food cups were put in. Cups containing test food but not exposed to birds were placed in vacant cages to determine mass changes due to moisture. Aluminum trays suspended from test cages under each cup caught spillage. After 3 h, the test food was removed and the birds' maintenance food replaced. The contents of test food cups were weighed and consumption determined by subtraction after appropriate adjustments for spillage and moisture gain or loss. Following each day's feeding trial, seed was separated from hulls and the latter stored in labeled plastic bags by species for eventual determination of fipronil residue. Hulls were manually separated from 100 rice seeds and weighed to determine the proportion of the seed mass represented by the hull. After the final treatment day, test birds were reweighed, banded, and released. Consumption by each species was analyzed in separate repeated measures analyses of variance, with treatment level as the between-subjects factor and day as the within-subjects factor.

Selected individuals were video-taped to document behavioral changes induced by feeding on fipronil-treated seeds. In particular, the tapes were examined for indications of illness (e.g. lack of activity, vomiting) or discomfort (e.g. pronounced head-shaking, gaping, excessive bill-wiping).

2.3 Enclosure trials

Three untested male redwings were placed into each of eight test enclosures (3·1 × 9·5 × 2·1 m). Each enclosure had shaded perches, a waterer, and two covered feeding stations. During the first four days both stations held a bowl of commercial game bird food. Then one bowl to hold 100 g of fipronil-treated rice and the other to hold 100 g of dyed, untreated rice were assigned at random. Each bowl rested on an aluminum spillage pan. During each of four test days, maintenance food was removed at 0700, and test food bowls presented at 0800. Separate food bowls, not exposed to birds, were put out to determine mass change due to ambient conditions. Test bowls and spillage pans were removed at 1500 and the maintenance food replaced. Contents of food bowls and pans were weighed and consumption determined by subtraction after correction for moisture gain or loss. Separate series of trials were conducted for the 325 mg kg^{-1} and 500 mg kg^{-1} fipronil treatment levels. Repeated measures analysis of variance tested for differences between treatment levels and among test days.

2.4 Flight pen trials

One of two 12 × 12 m plots within a 0·2-ha flight pen[9] was randomly selected to hold fipronil-treated seed and the alternate feeding plot was provisioned with untreated rice. We used six groups of 10 male red-winged blackbirds each. For groups 1–3, the untreated rice was dyed identically to the fipronil-treated rice. Groups 4–6 received undyed, untreated rice as the alternative. Sufficient birds were not available to conduct trials with undyed fipronil-treated rice and dyed control rice. Eight hundred grams of rice was hand-broadcast on each plot. Twenty sampling quadrats (0·2 m^2 each) were randomly located within each test plot, the initial seed count in each sampling quadrat adjusted to 50, and the seeds on the quadrats recounted each morning at 10:00. Seed removal was compared between plots in one-way analyses of variance. Each trial lasted four days. Birds were banded, weighed, and put into the flight pen on Monday morning. Daily observations were made during 08:00–10:00 from a blind at the north end of the flight pen, and locations of the birds were recorded at 5-min intervals. Use of test plots was compared in repeated measures analysis of variance. Test birds were trapped on Friday morning, reweighed and released, and the plots prepared for the next group.

2.5 Analytical chemistry

Rice seed samples were weighed into individual 25-ml glass tubes. A 10·0 ml aliquot of acetonitrile was added to each glass tube and sealed. The samples were centrifuged 5–10 s, then placed in a mechanical shaker on high speed for 10 min. The samples were sonicated for 30 min and then centrifuged for 2 min. The extract was then filtered through a 0·45-μm Teflon syringe filter into a sample vial and sealed. The sample extracts were analyzed by reversed-phase high-performance liquid chromatography with ultra-violet detection. The fipronil concentration was determined by comparing the area of the fipronil peak in the sample extract to a working standard.

Control rice seed and rice seed hull samples were fortified at the expected fipronil levels with aliquots of concentrated standards of fipronil in acetonitrile. The levels chosen for fortification of the control rice seed were 325 and 500 mg kg^{-1} fipronil. Rice seed hull samples were hypothesized to retain most of the fipronil after feeding by the granivorous birds. Therefore, the concentration of fipronil on the rice seed hull samples would probably be considerably greater than on the fortified whole rice seed samples. Thus, the levels chosen for fortification of the control rice seed hulls were 1390, 1630, and 2000 mg kg^{-1} fipronil. To compare fipronil residues on hulls with those on whole seeds, the hull residue was multiplied by 0·19, the proportion of whole rice seed mass represented by the hull (M. L. Avery, unpublished data).

3 RESULTS

3.1 One-cup feeding trials

Consumption of treated rice by individually caged brown-headed cowbirds did not vary ($F = 0{\cdot}02$; 2,15 df; $P = 0{\cdot}98$) between fipronil levels (Table 1) or across days ($F = 1{\cdot}47$; 3,45 df; $P = 0{\cdot}24$). Similarly, seed consumption by male red-winged blackbirds did not vary with fipronil level ($F = 0{\cdot}96$; 2,15 df; $P = 0{\cdot}41$) or with test day ($F = 0{\cdot}26$; 3,45 df; $P = 0{\cdot}86$). Mean daily consumption of treated rice by female boat-tailed grackles varied among treatments ($F = 4{\cdot}55$; 2,15 df; $P = 0{\cdot}03$) and days ($F = 5{\cdot}00$; 3,45 df; $P = 0{\cdot}004$). Lowest mean consumption occurred in the 0 mg kg^{-1} fipronil group (2·43 g per bird, SE = 0·38) and was due to two birds that did not eat the dyed seed. Mean consumption was lowest on treatment day 1 (2·92 g per bird, SE = 0·50), and highest on day 4 (4·52 g per bird, SE = 0·40).

Video-taped observations of test birds revealed no evidence of ill effects. Throughout each 2-h taped session, birds ate rice seed repeatedly and did not manifest behavior usually associated with illness or discomfort such as gagging, vomiting, or excessive head-shaking. Body mass changes among brown-headed cowbirds ranged from a mean gain of 0·9 g per bird (SE = 0·2) in the 500 mg kg^{-1} group to a mean gain of 1·2 g (SE = 0·6) in the 0 mg kg^{-1} group. Red-winged blackbirds gained an average of from 1·5 g per bird (SE = 0·7) in the 500 mg kg^{-1} group to 2·3 g per bird (SE = 0·4) in the 325 mg kg^{-1} group. Body mass change in boat-tailed grackles ranged from a mean increase of 8·1 g (SE = 5·0) in the 500 mg kg^{-1} group to a mean loss of 0·3 g (SE = 0·7) in the 0 mg kg^{-1} group.

TABLE 1
Mean Consumption during 3-h Feeding Trials across Four Days by Individually Caged Blackbirds Exposed to Rice Treated with Different Levels of Fipronil

	Rice consumed (g) (±SE)[a]		
	Fipronil level (mg kg^{-1})		
Species	*0*	*325*	*500*
Brown-headed Cowbird	2·22 (±0·14)	2·29 (±0·16)	2·28 (±0·19)
Red-winged Blackbird	3·40 (±0·33)	3·86 (±0·14)	4·39 (±0·23)
Boat-tailed Grackle	2·43 (±0·38)	4·20 (±0·34)	4·49 (±0·35)

[a] Six birds per species per level.

3.2 Enclosure trials

Rice consumption did not differ between treatment levels ($F = 1{\cdot}28$; 1,14 df; $P = 0{\cdot}28$) or among days ($F = 0{\cdot}08$; 3,42 df; $P = 1{\cdot}28$). Birds appeared indifferent to the presence of fipronil (Fig. 1). Daily consumption by the 325 mg kg^{-1} groups averaged 6·9 g (SE = 0·7) from the treated bowl and 7·2 g (SE = 0·8) from the alternate food bowl. Comparable values for the 500 mg kg^{-1} groups were 6·9 g (SE = 0·8) and 7·2 g (SE = 0·7), respectively.

3.3 Flight pen trials

When the alternate plot contained dyed, untreated rice, estimated seed removal was 12·5% (SE = 1·3%), compared to 11·4% (SE = 4·0%) from the fipronil-treated plot ($F = 0{\cdot}06$; 1,4 df; $P = 0{\cdot}81$). In contrast, when the alternate plot held undyed, untreated rice seed, estimated removal was 28·9% (SE = 5·3%), compared to just 2·4% (SE = 2·0%) from the treated plot ($F = 21{\cdot}87$; 1,4 df; $P = 0{\cdot}009$). A similar trend was apparent in observed bird use of the test plots (Fig. 2). Overall, the mean number of bird-minutes in the alternate plot (15·2, SE = 3·1) exceeded ($F = 9{\cdot}58$; 1,40 df; $P = 0{\cdot}004$) that in the treated plot (4·8, SE = 1·8). When the alternate plot contained dyed seed, use of the treated plot averaged 9·3 bird-minutes (SE = 3·1) compared to 0·2 bird-minutes (SE = 0·2) when undyed rice was in the alternate plot ($F = 2{\cdot}28$; 1,40 df; $P = 0{\cdot}14$). Body mass changes among groups of test birds ranged from a mean gain of 0·4 (SE = 1·0) in group 2 to a mean loss of 5·3 g (SE = 0·8) in group 4.

3.4 Chemical analyses

Mean recovery from the quality control samples was 95·9% (SE = 2·9%). Assays of the 325 and 500 mg kg^{-1} fipronil-treated rice seed revealed measured concentra-

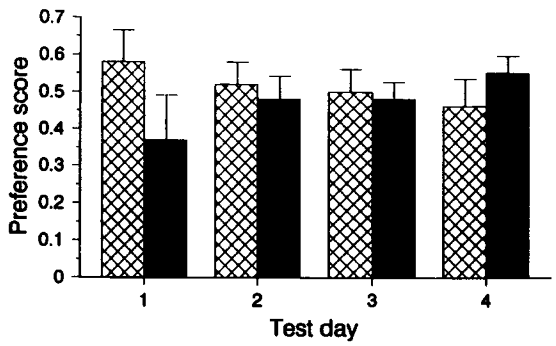

Fig. 1. Mean preference scores (consumption of fipronil-treated rice divided by total rice consumption) by groups of three red-winged blackbirds (eight groups per treatment level) in 3·1 × 9·5 × 2·1 m test enclosures during daily 7-h feeding trials on four consecutive days. A score of 0·5 indicates indifference to the fipronil treatments (▩) 325 mg kg^{-1} and (■) 500 mg kg^{-1}. Capped bars denote one SE.

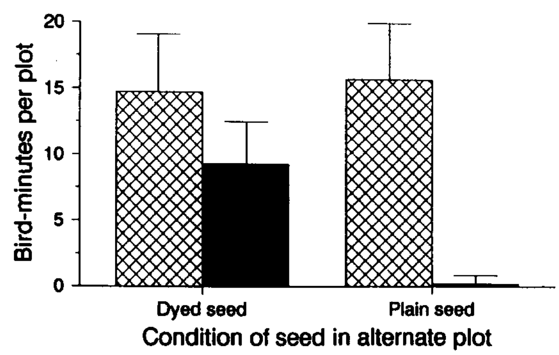

Fig. 2. Mean use of test plots by six groups of 10 red-winged blackbirds within a 0·2-ha flight pen during 08:00–10:00 on four consecutive mornings. One plot held (■) fipronil-treated rice seed, and the other (▩) untreated rice seed that was either dyed (three groups of birds) or undyed (three groups). Capped bars denote one SE.

TABLE 2
Fipronil residue on Rice Seed Hulls after Feeding by Three Species of Birds during Three-Hours trials with Different Levels of Fipronil, and Calculated Estimates of Fipronil Removed by Birds during Feeding

Species	*Fipronil on rice hulls* ($mg\ kg^{-1}$) ($\pm SE$)			Removed[b] (%) ($\pm SE$)
	Nominal	*Measured*	*Adjusted*[a]	
Brown-headed Cowbird	325	1348 (±36)	256 (±7)	11·4 (±2·4)
	500	1815 (±98)	345 (±19)	19·3 (±4·4)
Red-winged Blackbird	325	1365 (±33)	260 (±7)	10·2 (±2·3)
	500	1897 (±101)	361 (±19)	15·6 (±4·5)
Boat-tailed Grackle	325	1267 (±99)	241 (±19)	16·7 (±6·6)
	500	1820 (±81)	346 (±15)	19·0 (±3·6)

[a] Obtained by multiplying the measured amount of fipronil on the hulls by 0·19, the proportion of the whole seed mass represented by the hull.
[b] Calculated based on the measured amounts of fipronil initially present on the whole seed, 289 and 427 $mg\ kg^{-1}$, respectively, rather than the nominal levels.

tions of 289 (SE = 12, $n = 9$) and 427 $mg\ kg^{-1}$ (SE = 24, $n = 6$), respectively. Hulls collected from the one-cup feeding trials contained corresponding mean fipronil concentrations of 1332 and 1844 $mg\ kg^{-1}$, respectively. When these residues are corrected for the proportion of the seed mass represented by the hull (19%), the normalized fipronil concentrations are 253 (SE = 10) and 350 $mg\ kg^{-1}$ (SE = 10), respectively. Thus, during feeding, birds removed 10% to 20% of the fipronil originally present on the rice seeds (Table 2).

4 DISCUSSION

We found no indication that fipronil applied to rice seeds affected the birds' response to the seeds. The application rates tested, 325 and 500 $mg\ kg^{-1}$, were developed for insect pest control, not for avian feeding deterrence. The concentration at which fipronil affects avian feeding behavior is not presently known.

The flight pen trials confirmed that under certain conditions birds will avoid unusual or inappropriately colored food items.[10,11] The presence of normal-looking alternative food resulted in substantially reduced consumption of fipronil-treated rice. Conversely, with no alternative food (one-cup cage tests), or with visually identical alternative food (enclosure tests, flight pen trial), rice consumption was not reduced. If birds have a familiar, palatable alternative food, they will refrain from eating dyed food, which raises the possibility that certain hues can help reduce the likelihood of accidental ingestion of toxic pesticides.[12]

Analysis of whole seeds and of rice hulls that birds discarded showed that birds removed 10–20% of the fipronil applied to the seed. This is consistent with previous findings with blackbirds feeding on rice seed treated with imidacloprid, another insecticide.[7,13] One explanation for the lack of response by the birds to the fipronil treatment is that they were not exposed to sufficient quantities of the chemical. A different formulation that would permit greater amounts to be removed from the seed hull and ingested by birds might produce different responses.

In a 3-h feeding session, redwings and grackles ate 4–5 g of rice treated with 500 $mg\ kg^{-1}$ of fipronil (Table 1). Thus, if all of the chemical from 5 g of rice was ingested, these birds would receive 2·5 mg of fipronil in 3 h. Given that 15–20% of the fipronil was removed during feeding, however, maximum ingestion was approximately 0·5 mg. Under these test conditions, we detected no adverse effects to the birds, and we would expect similar results in the field.

ACKNOWLEDGEMENTS

Support for this study was provided by Rhône-Poulenc Ag Company and by the National Wildlife Research Center. C. L. Schreiber assisted with data collection and analysis, and K. L. Roca and C. C. McClester cared for the captive birds. R. K. Craver and D. L. Griffin assisted with chemical analyses, and J. L. Belant provided review comments. All birds were captured, maintained and tested according to procedures approved by the Animal Care and Use Committee of the National Wildlife Research Center.

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